PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 20347WO	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/08507	International filing date *(day/month/year)* 31.07.2003	Priority date *(day/month/year)* 06.08.2002
International Patent Classification (IPC) or both national classification and IPC C12N9/02		
Applicant DSM IP ASSETS B.V.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02.03.2004	04.11.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Piret, B Telephone No. +31 70 340-1966

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/08507

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-6, 8-22	as originally filed
7	received on 13.08.2004 with letter of 13.08.2004

Claims, Numbers

1-10	received on 13.08.2004 with letter of 13.08.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4,6-8,10
	No:	Claims	1-3,5,9
Inventive step (IS)	Yes:	Claims	10
	No:	Claims	1-9
Industrial applicability (IA)	Yes:	Claims	1-10
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: DATABASE CA [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; NAKAYAMA, KIYOSHI ET AL: 'Fermentative production of.beta.- (3,4-dihydroxyphenyl)-L-alanine' retrieved from STN Database accession no. 83:7116 XP002224427 & JP 49 100290 A (KYOWA HAKKO KOGYO CO., LTD.) 21 September 1974

D2: US-A-5 837 504 (LEE JANG YOUNG ET AL) 17 November 1998

D3: GERIGK M R ET AL: 'Enhanced pilot-scale fed-batch L-phenylalanine production with recombinant Escherichia coli by fully integrated reactive extraction.' BIOPROCESS AND BIOSYSTEMS ENGINEERING, vol. 25, no. 1, April 2002), pages 43-52, XP002224425; ISSN: 1615- 7591

D4: XUN LUYING ET AL: 'Characterization of 4-hydroxyphenylacetate 3-hydroxylase (HpaB) of Escherichia coli as a reduced flavin adenine dinucleotide-utilizing monooxygenase.' APPLIED AND ENVIRONMENTAL MICROBIOLOGY., vol. 66, no. 2, February 2000, pages 481-486, XP002224426 ISSN: 0099-2240

D5: BONGAERTS J ET AL: 'Metabolic engineering for microbial production of aromatic amino acids' METABOLIC ENGINEERING, ACADEMIC PRESS, US, vol. 3, October 2001, pages 289-300, XP002195863 ISSN: 1096-7176

1. Novelty (Article 33(1) and (2) PCT)

1.1. D1 discloses a process for the production of L-DOPA by aerobic culture of a *Pseudomonas* micro-organism in a medium at pH 7.0, using glucose as a carbon source, where the pH is adjusted to 6.0, and L-DOPA is isolated from the culture supernatant by adsorption onto Dowex-50 and elution with HCl. Since this micro-organism is capable of producing L-DOPA, it is assumed to be able to produce L-tyrosine and to possess an enzyme with L-tyrosine-3-hydroxymono-oxygenase activity such as L-tyrosinase or L-tyrosine hydroxylase. Therefore, the subject-matter of claims 1-3, 5 and 9 is not new.

2. Inventive step (Article 33(1) and (3) PCT)

2.1. The subject-matter of claim 4 differs from D1, which is the most relevant prior art, by the fact that a hydrophobic resin is used for the separation of L-DOPA instead of an ion-exchanger (Dowex-50) resin. The problem to be solved is: to provide a further process for L-DOPA production, using an alternative extraction process. The solution provided by claim 4 is to use hydrophobic chromatography. However, the use of a hydrophobic column (C18) for the isolation of L-DOPA in a very similar process is already disclosed in D2 (see Example 1). It would be obvious to use the isolation method of D2 with the process of D1 in order to provide a further process, identical to the one of claim 4. Therefore, the subject-matter of claim 4 does not involve an inventive step.

2.2. The subject-matter of claim 6 differs from D1 by the fact that it uses a process for the isolation of L-DOPA comprising pumping, filtering and reactive extraction. Again, the problem to be solved is: to provide an alternative process for L-DOPA production using an alternative extraction process. The solution provided in claim 6 (use of filtering and reactive extraction) cannot be considered as inventive, since it was already used for solving a very similar problem (i.e. the isolation of L-phenylalanine, a compound structurally similar to L-DOPA, from a fermentation medium), as shown in D3 (see p.45, right-hand column, 3rd paragraph). It would therefore be obvious for the person skilled in the art to combine this process with the fermentation process of D1 in order to arrive at the process according to claim 6. Therefore, the subject-matter of claim 6 does not involve an inventive step.

2.3. The subject-matter of claim 7 differs from D1 by the fact that it uses micro-organisms overexpressing 4-hydroxyphenylacetate 3-hydroxylase. Since no technical effect is caused by this difference, the problem to be solved is: to provide an alternative process for the production of L-DOPA. The solution proposed is: to use organisms expressing 4-hydroxyphenylacetate 3-hydroxylase for the conversion of L-tyrosine into L-DOPA during the fermentation. However, the use of micro-organisms overexpressing 4-hydroxyphenylacetate 3-hydroxylase for the fermentative production of L-DOPA from L-tyrosine is disclosed in D2 (column 2, first paragraph). It would therefore be obvious for the person skilled in the art to modify the micro-organisms of D1, using the teachings of D2 (paragraph bridging columns 2 and 3; Example 3) in order to obtain recombinant micro-organisms overexpressing 4-hydroxyphenylacetate 3-hydroxylase, thereby arriving at the process according to claim 7. Therefore, the subject-matter of claim 7 does

not involve an inventive step.

2.4. The 4-hydroxyphenylacetate 3-hydroxylase encoded by the *HpaB* gene is known (from D4, which would be known to the person skilled in the art) to be also capable of converting tyrosine to L-DOPA. It is known to use $FADH_2$ which is produced by a "$FADH_2$-NAD oxidoreductase" enzyme such as the *Fre* gene product (whereas the product of *HpaC* is believed to have the same activity). Therefore it would be obvious to overexpress said *Fre* (or *HpaC*) gene in combination with the *HpaB* gene in the microorganism of D1 in order to enhance the production of L-DOPA in the process of D1. For this reason the subject-matter of claim 8 does not involve an inventive step either.

2.5. It appears that some technical features essential for the definition of the invention, i.e. features that distinguish the process described in the application from the process of D1, and which result in advantages over said process of D1, are missing in the claims. More particularly, whereas the description alleges that an antioxidant is not required to prevent the degradation of L-DOPA in the claimed process, the technical features of said process that allow the omission of the antioxidant are not mentioned in claim 1.

2.6. Although the prior art (for instance D5) indicated the importance of the *tyrA* and *aroF* genes for the production of L-tyrosine, the precursor of L-DOPA, it did not clearly indicate that modifying the L-tyrosine biosynthetic pathway at these two stages would be sufficient to confer to an *E.coli* micro-organism such as the one used in D2 the ability to produce L-DOPA without addition of tyrosine. The prior art did not suggest to combine such a modification with the overexpression of *hpaB* and *hpaC* either. Therefore, an inventive step can be acknowledged for the subject-matter of claim 10.

3. Remarks regarding clarity (Article 6 PCT)

3.1. The term "$FADH_2$-NAD-oxidoreductase" used in claim 8 has no well-recognized, unambiguous meaning and leaves the reader in doubt as to the meaning of the technical features to which it refers (i.e. the chemical reaction(s) catalysed by the enzyme). It is misleading because it suggests that it refers to an enzyme that reduces NAD^+, using FADH2 as the electron source, whereas it is clear that HpaC mentioned in the description p.7 uses $NADH+H^+$ in order to reduce FAD. D4 cited p.7 in the description uses indeed the term "NAD(P)H:FAD

oxidoreductase". Therefore the term "$FADH_2$-NAD-oxidoreductase" renders claim 8 unclear.